

TOTAL ANTIOXIDANT CAPACITY AND POLYPHENOL CONTENT CORRELATION IN AQUEOUS-ALCOHOLIC PLANT EXTRACTS USED IN PHYTOTHERAPY

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ABSTRACT

The present investigation analyzes the antioxidant properties of aqueous-alcoholic extracts of Bulgarian plants used in phytotherapy in relation with their polyphenol content. The total phenolic (TP) content and total antioxidant capacity (TAC) values of aqueous-alcoholic extracts from *Hypericum perforatum*, *Cydonia vulgaris*, *Arctostaphylos uva-ursi* and *Cotinus coggygria* significantly exceeded the TP and TAC values of *Ilex paraguariensis* (mate), *Aspalathus linearis* (rooibos), *Cyclopia intermedia* (honeybush), and *Camellia sinensis* (black tea), famous for their antioxidants' content. The high degree of positive correlation ($r = 0,91$) between TAC and TP values indicates the role of polyphenols for the plant antioxidant potential. Bulgarian medicinal plants are excellent sources of antioxidants established by their high TAC values as compared to the famous foreign plants honeybush, rooibos, mate and black tea and they prove to be a potential for the development of new pharmaceuticals.

Key words: total antioxidant capacity, total phenolic content, Bulgarian medicinal plants, aqueous-alcoholic extracts

INTRODUCTION

Black tea, mate, honeybush and rooibos are popular nonalcoholic beverages with established high antioxidant potential related to high phenolic content. There is considerable interest in the isolation of more potent antioxidant compounds from natural sources.

Various methods and approaches have been applied to extract antioxidants from different plant materials and to evaluate their antioxidant capacity (1,3). It is difficult to compare data in the literature due to the lack of agreement on a reference method to analyze antioxidant capacity (4). Moreover, antioxidant activity often varies depending on the extraction procedure. Pharmaceutical plant-derived formulations include alcoholic extracts in the forms of tinctures and unguents.

The knowledge of Bulgarian medicinal plants' antioxidant potential is still in infancy. The objective of the present investigation was to analyze antioxidant properties of aqueous alcoholic extracts of Bulgarian plants used in phytotherapy and established a relationship between antioxidant capacity and their polyphenol content.

MATERIAL AND METHODS

Selection of plants

Nineteen of the most popular Bulgarian medicinal and four foreign plants (Table 1) used as tea beverages and in phytotherapy were selected on the basis of the antioxidant potential of their water extracts established in a previous study (2). Plants tested were in sale with no need for medical prescription and standardized according to the European Pharmacopoeia.

Plant extracts

Plant extracts were prepared as described by (8) with modifications. Dried herbs were blended into a fine powder and extracted three times (3 min each with continuous stirring at room temperature) with 80% (v/v) methanol/water (herb:methanol = 1:20 w/v). The supernatants from each step were combined and diluted to 25 mL with 80% methanol. Future dilutions (by 20 and 40 times) were prepared with 80% methanol. Total antioxidant capacity (TAC) and total phenolic (TP) content of the aqueous-methanolic plant extracts were measured.

TP content

Total phenolic (TP) content of aqueous-methanolic plant extracts was assessed by using the Folin-Ciocalteu phenol reagent as described in (7). Quercetin in methanol was used as a standard and TP content was expressed in μM .

Measurement of TAC

TAC of aqueous-methanolic plant extracts was determined by the ABTS (2,2'-azinobis (3-ethylbenzothiazoline-

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6-sulfonic acid)) radical decolorization assay (5). Alpha-tocopherol in methanol was used as a standard and TAC was expressed in mM.

Table 1. Total antioxidant capacity (TAC) and total polyphenol (TP) content of aqueous-methanolic plant extracts

Plant	TAC mean \pm SD (mM)	Total phenols mean \pm SD (μ M)
<i>Taraxacum officinale</i> complex	0,03 \pm 0,04	33,21 \pm 5,99
<i>Capsella bursa-pastoris</i> (L.) Medik.	0,03 \pm 0,01	10,60 \pm 0,87
<i>Lavandula angustifolia</i> Mill.	0,09 \pm 0,02	32,22 \pm 1,44
<i>Pulmonaria officinalis</i> L.	0,10 \pm 0,01	51,69 \pm 1,16
<i>Matricaria chamomilla</i> L.	0,10 \pm 0,01	39,88 \pm 1,15
<i>Cyclopia intermedia</i> E. Mey.	0,11 \pm 0,01	49,31 \pm 0,54
<i>Mentha piperita</i> L.	0,12 \pm 0,01	48,63 \pm 1,16
<i>Humulus lupulus</i> L.	0,13 \pm 0,01	40,80 \pm 0,87
<i>Aspalathus linearis</i> (N.L. Burm.) R. Dahlgr.	0,13 \pm 0,02	56,58 \pm 0,87
<i>Thymus sp. diversa</i>	0,19 \pm 0,05	67,80 \pm 0,87
<i>Ilex paraguariensis</i> A. St.-Hil.	0,20 \pm 0,03	112,45 \pm 0,66
<i>Crataegus monogyna</i> Jacq.	0,24 \pm 0,01	63,86 \pm 0,35
<i>Melissa officinalis</i> L.	0,29 \pm 0,06	128,54 \pm 2,01
<i>Ocimum basilicum</i> L.	0,34 \pm 0,02	91,23 \pm 2,56
<i>Tilia platyphyllos</i> Scop.	0,35 \pm 0,03	70,07 \pm 0,96
<i>Origanum vulgare</i> L.	0,42 \pm 0,02	99,65 \pm 0,46
<i>Agrimonia eupatoria</i> L.	0,53 \pm 0,01	124,40 \pm 4,85
<i>Rubus sp. diversa</i>	0,53 \pm 0,03	83,56 \pm 0,61
<i>Camellia sinensis</i> Kuntze (black tee)	0,67 \pm 0,06	117,90 \pm 7,48
<i>Hypericum perforatum</i> L.	0,77 \pm 0,05	162,26 \pm 2,46
<i>Cydonia vulgaris</i> Pers.	0,88 \pm 0,01	147,47 \pm 1,05
<i>Arctostaphylos uva-ursi</i> (L.) Spreng.	1,15 \pm 0,05	198,37 \pm 1,44
* <i>Cotinus coggygria</i> Scop.	0,89 \pm 0,13	159,74 \pm 1,44

*TAC and TP content measured at 20 times dilution of all plant extracts with the exception of *C. coggygria* extract which was diluted 40 times

Statistical analysis

Results were presented as means \pm SD. Correlation analysis was carried out by GraphPad Prism software.

RESULTS AND DISCUSSION

Aqueous-methanolic extracts of Bulgarian medicinal plants were studied for TAC and TP content in comparison with four foreign plants famous for their antioxidant potential: *Ilex paraguariensis* A. St.-Hil. (mate), *Aspalathus linearis* (N.L. Burm.) R. Dahlgr. (rooibos), *Cyclopia intermedia* E. Mey. (honeybush), and *Camellia sinensis* O. Kuntze (black tea) (Table 1). Bulgarian medicinal plants were divided into two groups according to their aqueous-methanolic TAC: plants with lower than 0,1 mM TAC values and plants with TAC higher than 0,1 mM. Five Bulgarian plants species exhibited low TAC. Interestingly, eleven Bulgarian medicinal plants were with higher TAC values than honeybush, rooibos and mate and four Bulgarian plants were with TAC values higher even than that of black tea extracts (Table 1). Aqueous-methanolic extract of *Cotinus coggygria* exhibited very high TAC which could not be measured at 20 times dilution and its TAC value at 40 times dilution was determined to be 0,89 \pm 0,13 mM.

TP content of the plant extracts with low TAC values was measured to be below 50 μ M (Table 1), while TP content of plants with higher TAC values varied from 50 to 100 μ M with the exception of the higher TP values of *Melissa officinalis* (128,54 \pm 2,01) and *Agrimonia eupatoria* (124,40 \pm 4,85). TP content of *Camellia sinensis*, *Hypericum perforatum*, *Cydonia vulgaris*, and *Arctostaphylos uva-ursi* were significantly exceeding 100 μ M. TP content of the 40 times diluted aqueous-methanolic extract of *C. coggygria* was determined to be 159,74 \pm 1,31 μ M. Polyphenols constitute one of the most widely distributed compounds in plants and contribute to a great extent to their antioxidant potential. Besides their anti-inflammatory effect and their role as free radical scavengers they have also metal ion chelating effect, antimutagenic effect (4) and cancer prevention effect (6).

We carried out a correlation analysis with the aim to analyze the relation between the antioxidant properties of Bulgarian medicinal plants and their TP content. Very high degree of positive correlation ($r=0,91$) between TAC and TP values was established (Fig. 1). It indicated that TAC antioxidant potential of aqueous-methanolic extracts of Bulgarian medicinal plants was due to a great extent to their polyphenol content. Healing effect of some Bulgarian medicinal plants is, probably, due to their high polyphenol content.

CONCLUSIONS

1. Bulgarian medicinal plants are good sources of antioxidants established by their high TAC values as compared to the famous foreign plants honeybush, rooibos, mate and black tea.

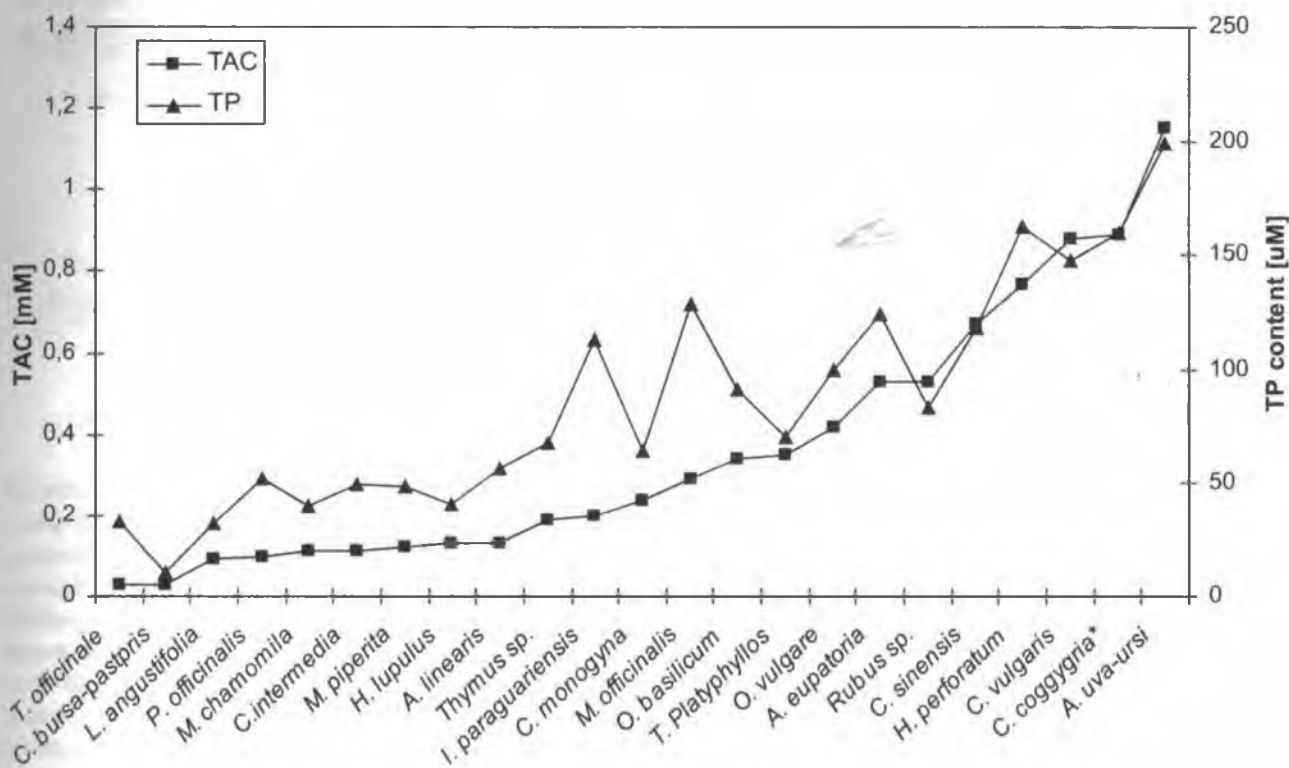


Fig. 1. Correlation between TAC and TP content of aqueous-methanolic plant extracts ($r=0,91$)

*TAC and TP content measured at 20 times dilution of all plant extracts with the exception of *C. coggygria* extract which was diluted 40 times

2. The high degree of positive correlation between TAC and TP values ($r=0,91$) indicates the role of polyphenols for the plant antioxidant potential.

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